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PLASMID, vol. 10, 1983, pages 21-30 L.COMAI et al.: "A new technique for genetic engineering of agrobacterium Ti plasmid."

Description

Technical Field

This invention is in the fields of genetic engineering, plant biology, and bacteriology.

Background Art

In the past decade, the science of genetic engineering has developed rapidly. A variety of processes are known for inserting a heterologous gene into bacteria, whereby the bacteria become capable of efficient expression of the inserted genes. Such processes normally involve the use of plasmids which may be cleaved at one or more selected cleavage sites by restriction endonucleases, discussed below. Typically, a gene of interest is obtained by cleaving one piece of DNA and the resulting DNA fragment is mixed with a fragment obtained by cleaving a vector such as a plasmid. The different strands of DNA are then connected ("ligated") to each other to form a reconstituted plasmid. See, for example, U.S. Patents 4,237,224 (Cohen and Boyer, 1980); 4,264,731 (Shine, 1981); 4,273,875 (Manis, 1981); 4,322,499 (Baxter et al. 1982), and 4,336,336 (Silhavy et al. 1982). A variety of other reference works are also available. Some of these works describe the natural processes whereby DNA is transcribed into messenger RNA (mRNA) and mRNA is translated into protein; see, e.g., Stryer, 1981 (note: all references cited herein, other than patents, are listed with citations after the Examples); Lehninger, 1975. Other works describe methods and products of genetic manipulation; see, e.g., Maniatis et al, 1982; Setlow and Hollaender, 1979.

Most of the genetic engineering work performed to date involves the insertion of genes into various types of cells, primarily bacteria such as E. coli, various other types of microorganisms such as yeast, and mammalian cells. However, many of the techniques and substances used for genetic engineering of animal cells and microorganisms are not directly applicable to genetic engineering involving plants.

As used herein, the term "plant" refers to a multicellular differentiated organism that is capable of photosynthesis, such as angiosperms and multicellular algae. This does not include microorganisms, such as bacteria, yeast, and fungi. However, the term "plant cells" includes any cell derived from a plant; this includes undifferentiated tissue such as callus or crown gall tumor, as well as plant seeds, propagules, pollen; and plant embryos.

A variety of plant genes have been isolated, some of which have been published and or are publicly available. Such genes include the soybean actin gene (Shah et al 1982), corn zein (Pederson et al. 1982) soybean leghemoglobin (Hyldig-Nielsen et al. 1982), and soybean storage proteins (Fischer and Goldberg, 1982).

The Regions of a Gene

The expression of a gene involves the creation of a polypeptide which is coded for by the gene. This process involves at least two steps: part of the gene is transcribed to form messenger RNA, and part of the mRNA is translated into a polypeptide. Although the processes of transcription and translation are not fully understood, it is believed that the transcription of a DNA sequence into mRNA is controlled by several regions of DNA. Each region is a series of bases (i.e., a series of nucleotide residues comprising adenosine (A), thymidine (T), cytidine (C), and guanidine (G)) which are in a desired sequence. Regions which are usually present in a eucaryotic gene are shown on Figure 1. These regions have been assigned names for use herein, and are briefly discussed below. It should be noted that a variety of terms are used in the literature, which describes these regions in much more detail.

An association region 2 causes RNA polymerase to associate with the segment of DNA. Transcription does not occur at association region 2; instead, the RNA polymerase normally travels along an intervening region 4 for an appropriate distance, such as about 100-300 bases, after it is activated by association region 2.

A transcription initiation sequence 6 directs the RNA polymerase to begin synthesis of mRNA. After it recognizes the appropriate signal, the RNA polymerase is believed to begin the synthesis of mRNA an appropriate distance, such as about 20 to about 30 bases, beyond the transcription initiation sequence 6. This is represented in Figure 1 by intervening region 8.

The foregoing sequences are referred to collectively as the promoter region of the gene.

The next sequence of DNA is transcribed by RNA polymerase into messenger RNA which is not

CHEMICAL ABSTRACTS, vol. 101, December 18, 1984, page 149, ref. no. 205249p; Columbus, Ohio, US R.T.FRALEY et al.: "Use of a chimeric gene to confer antibiotic resistance to plant cells."

JOURNAL OF MOLECULAR AND APPLIED GENETICS, no. 6, 1982, pages 561-573; Raven press, New York, US A.DEPICKER et al.: "Nopaline synthase: Transcript mapping and DNA sequence."

SCIENCE, vol. 214, December 4, 1981, pages 1133-1135 S.T.KELLOGG et al.: "Plasmid assisted molecular breeding: New Technique for enhanced biodegradation of persistent toxic chemicals."

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XI. **PAYMENT OF THE OPPOSITION FEE is made**

[X] as indicated in the enclosed voucher for settlement of fees (EPO Form 1010)

[ ]

XII. **LIST OF ENCLOSED DOCUMENTS**

Enclosure No.			No. of copies	
0	[X]	Copies of this form for notice of opposition	1	(min. 1)
1	[X]	Facts and arguments (see VII.)	2	(min. 2)
2	[X]	Separate indication of further evidence (see X)	2	(min. 2)
		Copies of documents presented as evidence (see X):		
3a	[X]	– Publications	2	(min. 2 of each)
3b	[ ]	– Other documents		(min. 2 of each)
4	[X]	Signed authorisation(s) (see IV)		
5	[X]	Voucher for the settlement of fees (see XI)		
6	[ ]	Cheque		
7	[ ]	Additional sheet		(min. 2)
8	[ ]	Receipt for documents (EPO Form 1037)	2	
9	[X]	Other (please specify here) Further opponents	1	

XIII. **SIGNATURE of opponent or representative**

Ernest GUTMANN
Professional representative
before the EPO

Place Paris,

Date December 4th, 1991

Please type name under signature. In the case of legal persons, the position of the signer within the company should also be typed.

X. **EVIDENCE presented**

for EPO only

A **Publications:**

(cited in patent specification, therefore not enclosed = 2)

(enclosed = 1)

(neither cited in patent specification nor enclosed = 0)

Date of public./ available (R.59)

1 Relates to claim(s) No. (see Annex 1)

Groneborn, et al., "Propagation of Foreign DNA in Plants Using Cauliflower Mosaic Virus as Vector", Nature, vol 294, 24/31 December, pp. 773-776

Particular relevance (page, column, line, fig.):

(see Annex 1)

2 Relates to claim(s) No. (see Annex 1)

Olszewski, et al, "A Transcriptionally Active, Covalently Closed Minichromosome of Cauliflower Mosaic Virus DNA Isolated from Infected Turnip Leaves", Cell, vol. 29, pp395-402, June, 1982.

Particular relevance (page, column, line, fig.):

(see Annex 1)

3 Relates to claim(s) No. (see Annex 1)

McKnight, et al, "Isolation and Mapping of Small Cauliflower Mosaic Virus DNA Fragments Active as Promoters in Escherichia coli", J. Virol. Feb 1981, pp673-682.

Particular relevance (page, column, line, fig.):

(see Annex 1)

4 Relates to claim(s) No.

Particular relevance (page, column, line, fig.):

5 Relates to claim(s) No.

Particular relevance (page, column, line, fig.):

Continued on separate sheet (annex 2)

B. **Other evidence:**

Continued on separate sheet (annex 2)